Reversal of Photoschedule in Spring Does not Prevent Photorefractoriness in Siberian Hamsters

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ABSTRACT We studied the influence of light–dark (L:D) cycle reversal on daily variations in the brown adipose tissue (BAT) capacity for nonshivering thermogenesis (NST) in Siberian hamsters (Phodopus sungorus). Continuous and simultaneous measurements of BAT temperature ($T_{BAT}$) and preferred ambient temperature ($P_Ta$) were made after noradrenaline (NA) injections administered every 4 hr. First, hamsters were acclimated for 4 weeks to an ambient temperature ($T_a$) of 23°C and 12L:12D, and then to a reversed photoschedule 12D:12L for 8 weeks. The same was done after a 4- and 8-week acclimation period at the same $T_a$. We found that after photoschedule reversal, the re-entrainment of $T_{BAT}$ and $P_Ta$ rhythms preceded re-entrainment of the NST rhythm. The daily rhythms of $T_{BAT}$ and $P_Ta$ were fully re-entrained after 4 weeks of acclimation to the reversed photoschedule, but rhythmicity of the response to NA disappeared. This rhythm was restored in hamsters acclimated to a reversed photoschedule for 8 weeks. We suggest that the daily rhythm of NST capacity is not responsible for generating the rhythm of body temperature ($T_b$). Rather, it is a result of the daily rhythm of $T_b$, but adjusts to the new environment more slowly than the $T_b$ rhythm. When a daily rhythm of NST was present, the increase in $T_{BAT}$ after NA injection was inversely correlated with the pre-injection $T_{BAT}$. In addition, NA-induced changes in $P_Ta$ reflected the intensity of NST in BAT; namely, increased $T_{BAT}$ was correlated with the post-injection decrease in $P_Ta$. When the increase in $T_{BAT}$ was large, animals chose a lower $T_a$ to dissipate excessive heat and prevent overheating. In the course of the experiments, we recorded a decreased mean NST capacity and increased body mass of hamsters. These changes are representative of the time of photorefractoriness and a transition to a summer status. Despite prolonged exposure to an intermediate day length (12 hr of light) and photoschedule reversal, hamsters continued to change towards their summer condition and were able to acclimate to the new D:L cycle. J. Exp. Zool. 303A:976–986, 2005. © 2005 Wiley-Liss, Inc.

Siberian hamsters (Phodopus sungorus) are small nocturnal rodents that exhibit a robust circadian rhythm of body temperature ($T_b$; Scribner and Wynne-Edwards, '94; Ross, '98; Ruf and Heldmaier, 2000). As in other nocturnal mammals, $T_b$ in hamsters is relatively high at night (i.e., in their active phase, $\alpha$; see Refinetti and Menaker ('92) for a review). Hamsters become active immediately or shortly after the lights are turned off, while the end of the $\alpha$ phase coincides with lights on. When at rest, hamsters maintain their body temperature at about 36°C. During the active phase, $T_b$ rises to temperatures above 37°C (Puchalski and Lynch, '86; Klante and Steinlechner, '95). An amplitude of daily variations in $T_b$ depends on photoperiod: in hamsters acclimated to a long day (Heldmaier et al., '89; Ruby and Zucker, '92).

The primary pacemaker for the regulation of circadian rhythms is situated in the suprachiasmatic nuclei (SCN) of the hypothalamus. Indeed, after bilateral lesion of the SCN, $T_b$ rhythm disappears (Ruby et al., '89). From the SCN, the photoperiodic information is transferred to the pineal gland and results in the melatonin signal. Regardless of the species’ activity pattern (diurnal or nocturnal), the melatonin level is higher during darkness (Saarela and Reiter, '93). The Siberian hamster is a highly photoperiodic rodent that

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strongly depends on changes in photoperiod to cue acclimatization, while ambient temperature has a minor effect (Ruf et al., '93). Under a short photoperiod, $T_b$ is lower than under a long photoperiod, but larger ultradian variations are recorded. Such changes in circadian organization of $T_b$ might allow for energy savings (Heldmaier et al., '85, '89). The reduction of energy requirements is of vital importance in a cold environment, which in the natural environment of hamsters usually coincides with short days.

In autumn, when the photoperiod shortens, hamsters are sensitive to short days (photosensitive) and prepare for winter: they lose body mass, increase nonshivering thermogenesis (NST) capacity, their fur changes to white, and they start to exhibit daily torpor and gonadal regression. In spring (or after 4–5 months under a short photoperiod), hamsters become photorefractory (i.e., insensitive to short days) and spontaneously return to their summer status. During the state of photorefractoriness, hamsters increase body mass, their fur changes to a grey one for summer and they exhibit gonadal recrudescence. Daily torpor is ceased and NST capacity is decreased to a lower, summer level (Hoffmann, '73; Heldmaier and Steinlechner, '81; Heldmaier et al., '81, '82; Heldmaier and Lynch, '86; Lynch and Puchalski, '86; Jefimow et al., 2004b). Seasonal changes in thermoregulation and behaviour ensure winter survival. In the Siberian hamster, as in many other small placental mammals, NST is a primary source of heat and plays a very important role in the maintenance of normothermy (Jansky, '73). In hamsters exposed to a short photoperiod, NST capacity is higher than in animals exposed to a long one (Heldmaier et al., '82). In addition, NST capacity also depends on the time of day (Jefimow et al., 2000, 2003). Also, in other rodents, such as spiny mice (Acomys rufus and Acomys cahirinus) and wood mice (Apodemus sylvaticus), daily variations in NST have been recorded (Kronfeld et al., '94; Haim et al., '95; Haim and Zisapel, '99). However, the basis of these variations is not yet well understood.

In small placental mammals, the major site for regulatory NST is brown adipose tissue (BAT; Jansky, '73; Nicholls and Locke, '84; Kuroshima, '93). Thus, temperature in the BAT can be used as an indicator of NST capacity (Hayward and Lyman, '67; Hayward, '68; Hashimoto et al., 2002; Jefimow et al., 2004a).

In the present study, we hypothesized that NST capacity depends on the actual level of $T_b$ and is strongly influenced by the prevalent light–dark cycle. We predicted that the rhythm of NST capacity, as related to the $T_b$ rhythm, would follow daily changes in $T_b$ that would occur as a result of adjustment to new photoperiodic conditions. Hence, after acclimation to a reversed photoschedule and re-entrainment of a $T_b$ rhythm, the daily rhythm of BAT capacity for regulatory NST should also be re-entrained. This would bring about higher NST capacity during the resting ($\rho$) phase and lower NST capacity during the active ($\alpha$) phase, independent of the photoperiodic regime. In addition, since the daily rhythm of preferred ambient temperature ($PT_\alpha$) opposes the daily rhythm of $T_b$ (Gordon, '94; Refinetti, '95), we predicted that the daily rhythm of $PT_\alpha$ would follow the re-entrainment of $T_b$ rhythm, being higher when $T_b$ is low and vice versa.

In order to test our predictions, we investigated the influence of $T_b$ (measured as interscapular $T_{BAT}$) rhythm on daily variations in BAT capacity for regulatory NST in Siberian hamsters acclimated to a 12L:12D photoschedule, and then to a reversed 12D:12L photoschedule. To test the thermogenic capacity of BAT, we administrated noradrenaline (NA) every 4 hr and continuously monitored $T_{BAT}$ and $PT_\alpha$. Since NA activates NST and induces hyperthermia (Jansky, '73), the magnitude of changes in BAT temperature after NA would reflect the intensity of NST. When NST is activated, hamsters would prefer lower ambient temperatures to facilitate the maintenance of normothermic body temperature (Gordon, '93).

**MATERIAL AND METHODS**

**Animals and housing**

Nine adult (4-month-old) male Siberian hamsters (P. sungorus) from our own breeding colony were used in this study. Before the start of the experiments in March, the animals were housed individually in standard laboratory cages (33 × 20 × 18 cm) under the natural photoperiod (12L:12D in March). At the start of the first set of experiments, the animals were moved to a climate chamber on a 12L:12D cycle (L:D cycle; lights on at 09:00), with an ambient temperature ($T_a$) of 23°C for 4 weeks. Food and water were available ad libitum. During the next 4 weeks, the hamsters were housed in the same climate chamber, but with a reversed photoschedule [i.e. 12D:12L (D:L cycle; lights on at 21:00 h)] at a $T_a$ of 23°C. After 8 weeks under the reversed photoschedule, the animals were sampled again. Photoschedule
reversal was accomplished by the prolongation of the light phase of the cycle; that is, on the day of reversal the hamsters experienced 24L:12D.

After acclimation to the L:D cycle, six out of nine animals were used. After 4 weeks of acclimation to D:L cycle, three previously used hamsters were replaced with new ones that were acclimated simultaneously, but were not used in the first set of experiments. This replacement was applied to evaluate whether injections during the first experiment could affect the results of the second one. After 8 weeks of acclimation to D:L cycle, six hamsters used in the previous experiments were used again.

To monitor changes in body mass (\(m_b\)), which are a good indicator of acclimation in Siberian hamsters (Hoffmann, '73), they were weighed to \(\pm 0.1\) g before experiment and then after acclimation to L:D and D:L cycles (i.e., every 4 weeks).

**Measurements of \(T_{BAT}\) and \(PT_a\)**

At least 3 days before an experiment, we implanted a polyethylene cannula (0.8 mm in diameter, 5 cm in length, SIMS Portex Ltd., Hythe, UK) in each hamster under Sevorane (Abbott Laboratories Ltd., Queensborough, UK) anaesthesia. The cannula was inserted under the skin, through a small incision (3 mm) at the back of the neck, and then fixed with surgical thread and adhesive to the skin. During the experiments, the cannula served as a guide for a copper-constantan thermocouple (0.6 mm in diameter, W-TW-36 P2; Physitemp Instruments Inc., Clifton, NJ, USA).

After acclimation to L:D and D:L cycles, each animal was tested individually in a thermal gradient system that allowed for continuous and simultaneous measurements of preferred ambient temperature (\(PT_a\)) and BAT temperature (\(T_{BAT}\)). The thermal gradient consisted of a long aluminium chamber (120 cm length \(\times\) 10 cm height \(\times\) 8 cm width), divided by half-width partitions into 16 compartments of the same size, and covered with transparent Perspex to permit light entry. The system was heated at one end and cooled at the other, resulting in a range of temperatures increasing linearly from 5°C to 45°C. The position of the animal, and thus its selected temperature, was detected at 1-sec intervals by infrared photodetector-photodetector pairs placed in each compartment. A narrow slit in the transparent lid allowed movement of the thermocouple that was suspended above the gradient by an elastic band. This design allowed hamsters to move freely inside the gradient without a load. \(T_{BAT}\) and \(PT_a\) were automatically recorded at 1-sec intervals and saved on disk.

**Experimental design**

**Response to NA**

Each animal was placed in the thermal gradient box for 3 days. On the first day, the thermocouple wire was inserted into the cannula to a depth where large BAT deposits are present. Then the hamster was left undisturbed to enable its habituation. On the next day, beginning at 09:00, NA [(\(\pm\)-Arterenol; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) at a dose of 0.6 mg kg\(^{-1}\) or saline (in the same volume as NA) was injected subcutaneously every 4 hr. Saline injections were a control for NA; while NA activates NST, changes in body temperature after saline reflect stress of injection. Night-time injections were done under dim red light. Half of the animals were injected with NA on the second day and then with saline on the third day. The sequence of injections in the other half of animals was reversed (first day—undisturbed, second day—saline, third day—NA). \(T_{BAT}\) and \(PT_a\) in saline and NA-injected hamsters were measured continuously. Temperatures before each injection were used to assess daily rhythms of \(T_{BAT}\) and \(PT_a\).

**Statistical analysis**

All recorded data were plotted at 10-min intervals as means ± SE. If a hamster chewed its lead shortly before or after injection, data collected for 1 hr after the repair were discarded. To assess daily rhythms of \(T_{BAT}\) and \(PT_a\), data collected 30 min before each injection were analysed.
assess the effects of saline and NA on $T_{\text{BAT}}$ and $PT_a$, data collected 30 min before (as a reference) and 60 min after each injection were compared. We found that the sequence of injections did not influence $T_{\text{BAT}}$ or $PT_a$ (two-way ANOVA, $P = 0.85$). There was no difference in the response to saline or to NA by hamsters that were used for the first time or re-tested (two-way ANOVA, $P = 0.32$). Changes in $T_{\text{BAT}}$ and $PT_a$, induced by injected pharmacological agents, are presented as the difference between mean $T_{\text{BAT}}$ or $PT_a$ recorded within 60 min after and 30 min before the injection ($\Delta T_{\text{BAT}}$; $\Delta PT_a$). To clarify the results, in the text below, $T_{\text{BAT}0}$ refers to BAT temperature before injection and $T_{\text{BAT}1}$ refers to BAT temperature after injection.

Changes in $T_{\text{BAT}0}$, $T_{\text{BAT}1}$ and $PT_a$ were analysed using three-way ANOVA to investigate the influence of photoschedule, type of injection and time of day. When ANOVA showed significant influence of the analysed factors, a post-hoc LSD test followed by a Bonferroni correction was used for comparison of means. The Pearson correlation coefficient, $r$, was used to test for correspondence between $T_{\text{BAT}0}$ and $PT_a$ as well as between $T_{\text{BAT}0}$ and $T_{\text{BAT}1}$. Changes in body mass ($m_b$) were analysed using one-way ANOVA followed by Tukey’s post-hoc test. Differences were considered statistically significant if $P < 0.05$. All values are presented as mean $\pm$ SE.

Experiments were approved by the Local Committee for the Ethics in Animal Research.

RESULTS

The effects of 4 weeks of acclimation to the reversed photoschedule

BAT temperature ($T_{\text{BAT}}$; Fig. 1)

After 4 weeks of acclimation to a reversed photoschedule (D:L cycle), the daily rhythm of $T_{\text{BAT}}$ before injection ($T_{\text{BAT}0}$) was fully reversed and entrained to a new D:L cycle (Fig. 1). A three-way ANOVA revealed the significant influence of time of day on $T_{\text{BAT}0}$ ($F_{(5,101)} = 7.06, P < 0.0001$), while type of injection and photoschedule had no effects. The magnitude of changes in $T_{\text{BAT}}$ after injection ($T_{\text{BAT}1}$) was significantly correlated with the type of injection, photoschedule and time of injection (three-way ANOVA: $F_{(5,1000)} = 2.95, P < 0.05$; Fig. 1). In general, NA induced a larger increase in $T_{\text{BAT}}$ than saline and this increase was larger in hamsters acclimated to L:D than to D:L cycle.

Fig. 1. Mean ($\pm$ SE) brown adipose tissue temperature ($T_{\text{BAT}}$) before each injection (—) and changes in $T_{\text{BAT}}$ ($\Delta T_{\text{BAT}}$; bars) induced by saline and noradrenaline (NA) injections in Siberian hamsters acclimated for 4 weeks to L:D cycle (panel A) and then after 4 and 8 weeks of acclimation to a reversed photoschedule (panels B and C, respectively). Dark bars at the top of each panel indicate time of darkness. Stars indicate significant differences between saline- and NA-injected hamsters: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Significantly different values within each group are indicated by the superscripts: a–b, c–d, e–f, g–h, i–j–k: $P < 0.05$; d–f: $P < 0.001$.

After acclimation to L:D cycle, daily variations in the response to NA and saline were also recorded; the largest increases in $T_{\text{BAT}1}$ after NA were recorded at CT 4, CT 8 and CT 20 and after saline at CT 4 and CT 12. After 4 weeks of acclimation to a reversed photoschedule (D:L cycle), daily variations in the response to saline or NA injections...
disappeared. However, the increase in \( T_{\text{BAT1}} \) after NA injection at CT 4, CT 8 and CT 20 was smaller than that after acclimation to L:D cycle \((P<0.001, 0.004 \text{ and } 0.001, \) respectively; Fig. 1). In saline-injected hamsters acclimated for 4 weeks to D:L cycle, the increase in \( T_{\text{BAT1}} \) at CT 4 and CT 12 was smaller than that after acclimation to L:D cycle \((P<0.008 \text{ and } 0.05, \) respectively), while at CT 0 the increase in \( T_{\text{BAT1}} \) was larger \((P<0.02)\).

**Preferred ambient temperature \( (PT_a; \) Fig. 2)**

After 4 weeks of acclimation to D:L cycle, the daily rhythm of preferred ambient temperature was also re-entrained. This temperature was significantly correlated with time of day \((\text{three-way ANOVA: } F_{(5,89)} = 2.96, P<0.05, \text{ Fig. 2})\) while type of injection and photoschedule were not correlated. The magnitude of changes in \( PT_a \) after injection was closely correlated with type of injection, photoschedule and time of day \((\text{three-way ANOVA: } F_{(5,92)} = 2.45, P<0.04; \text{ Fig. 2})\). NA always caused a more pronounced decrease in \( PT_a \) than saline, except for the injection at the end of night \((\text{CT } 20)\) in animals acclimated to D:L cycle.

We also recorded daily variations in \( PT_a \) after NA. In hamsters acclimated to L:D cycle, the time of day did not significantly affect the decrease in \( PT_a \) after NA injection; however, it varied from \(-4.2 \pm 0.5^\circ \text{C} \) to \(-8.0 \pm 1.0^\circ \text{C} \). After photoschedule reversal, preferred ambient temperature of saline-injected hamsters did not correlate with the time of day, while in NA-injected hamsters, the decrease in \( PT_a \) at CT 0, CT 4, CT 8 and CT 12 was larger than at CT 20 \((\text{Fig. 2})\).

The magnitude of changes in preferred ambient temperature depended on photoschedule. After acclimation to L:D cycle, the decrease in \( PT_a \) after NA injection at the beginning of the day \((\text{CT } 0)\) and the end of night \((\text{CT } 20)\) was larger than after acclimation to the reversed photoschedule \((P<0.05 \text{ and } 0.001, \) respectively). In saline-injected hamsters, differences were recorded at CT 12 and CT 20 \((P<0.03 \text{ and } 0.02, \) respectively; Fig. 2).

**Correlation between \( T_{\text{BAT0}}, T_{\text{BAT1}} \) and \( (PT_a; \) Table 1)**

In hamsters acclimated to L:D cycle, the magnitude of increase in BAT temperature after injection \((T_{\text{BAT1}})\) was inversely correlated with pre-injection temperature of BAT \((T_{\text{BAT0}});\) the higher the initial temperature, the smaller was the increase after injection. After photoschedule reversal, such a correlation was found only in saline-treated hamsters \((\text{Table 1})\). Before injection, \( T_{\text{BAT0}} \) was inversely correlated with \( PT_a \) only in animals acclimated to D:L cycle \((r = -0.49, \text{Fig. 2})\),
PHOTOSCHEDULE REVERSAL IN SIBERIAN HAMSTERS

TABLE 1. The increase in brown adipose tissue temperature (T_{BAT}) after noradrenaline (NA) and saline injections was inversely correlated with T_{BAT} before injection in Siberian hamsters acclimated to L:D cycle and then after 4 and 8 weeks of acclimation to a reversed photoschedule (D:L 4 and D:L 8, respectively)

<table>
<thead>
<tr>
<th>Photoschedule</th>
<th>Injection</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>L:D</td>
<td>NA</td>
<td>-0.56</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>-0.68</td>
<td>0.001</td>
</tr>
<tr>
<td>D:L 4</td>
<td>NA</td>
<td>-0.32</td>
<td>n.s. (0.08)</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>-0.40</td>
<td>0.03</td>
</tr>
<tr>
<td>D:L 8</td>
<td>NA</td>
<td>-0.79</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>-0.62</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Only in NA-injected hamsters acclimated for 4 weeks to D:L cycle, this correlation was not significant.

P < 0.001. Increase in T_{BAT1} after NA injection was correlated with decreased PT_{a}, both in animals acclimated to L:D cycle (r = 0.36, P < 0.001), as well as to D:L cycle (r = 0.48, P < 0.001). In saline-injected hamsters, significant correlation was found only after acclimation to D:L cycle (r = 0.32, P < 0.001).

**The effects of 8 weeks of acclimation to the reversed photoschedule**

**BAT temperature (T_{BAT}; Fig. 1)**

After 8 weeks of acclimation to the reversed photoschedule, the daily rhythm of T_{BAT0} was also fully reversed in comparison to L:D cycle (three-way ANOVA: F(5,99) = 5.30, P < 0.001, Fig. 1). BAT temperatures (T_{BAT0}) in saline- and NA-injected hamsters did not differ. The magnitude of changes in BAT temperature after injection (T_{BAT1}) was significantly correlated with type of injection, photoschedule and time of day (three-way ANOVA: F(5,98) = 2.32, P < 0.05; Fig. 1).

Overall, after 8 weeks of acclimation to D:L cycle, NA induced a much smaller increase in T_{BAT} than in animals acclimated to L:D cycle. Daily variations were also recorded. The largest increase in T_{BAT1} after NA injection was again recorded at the end of the day (CT 8), while the smallest increase was recorded at the end of night (CT 20). After the injections at CT 4, CT 8, CT 12 and CT 20, the increase in T_{BAT1} after NA was much smaller than that in hamsters acclimated to L:D cycle (P < 0.001, 0.05, 0.001 and 0.001, respectively; Fig. 1). After 8 weeks in D:L cycle, the response to saline was also smaller than in L:D cycle at CT 0 (P < 0.01), CT 4 (P < 0.001), CT 8 (P < 0.01), CT 12 (P < 0.01) and CT 20 (P < 0.05).

**Preferred ambient temperature (PT_{a}; Fig. 2)**

After 8 weeks of acclimation to D:L cycle, the daily rhythm of PT_{a} was also re-entrained (three-way ANOVA: F(5,93) = 2.49, P < 0.04; Fig. 2). Before injection, PT_{a} of saline- and NA-injected animals did not differ. Changes in PT_{a} after injection were closely correlated with type of injection, photoschedule and time of day (three-way ANOVA: F(5,100) = 3.62, P < 0.005; Fig. 2). In D:L cycle, NA injection at the beginning of night (CT 12) induced the largest decrease of PT_{a}, to a similar degree as that in L:D cycle. After other NA injections, changes in preferred ambient temperatures were smaller than in L:D cycle (P < 0.001). After 8 weeks under the reversed photoschedule, saline injections induced significant changes in PT_{a} only at CT 0 and CT 12 (Fig. 2). Significant differences in PT_{a} changes after saline injections, between hamsters acclimated to L:D and D:L cycles, were recorded at CT 0 (P < 0.001), CT 4 (P < 0.04), CT 12 (P < 0.001) and CT 20 (P < 0.001).

**Correlation between T_{BAT0}, T_{BAT1} and (PT_{a}; Table 1)**

In the hamsters acclimated to D:L cycles for 8 weeks, there was a strong inverse correlation between T_{BAT1} and T_{BAT0} (Table 1). T_{BAT0} was also inversely correlated with PT_{a} (r = -0.63, P < 0.001). The increase in T_{BAT1} induced by saline injections was correlated with a decrease in PT_{a} (r = 0.38, P < 0.001). In NA-injected animals, such a correlation was not recorded.

**Influence of photoschedule and total time of acclimation to 12 hr of light on T_{BAT1} and PT_{a} after saline and NA injections (Fig. 3)**

Changes in BAT temperature after injection (T_{BAT1}) during the 12 weeks of experiments were significantly correlated with photoschedule and type of injection (two-way ANOVA: F(2,176) = 9.84, P < 0.04; Fig. 3A). The mean increase in T_{BAT1} after NA injections in L:D cycle (mean for all injections 0.9 ± 0.1°C) was larger than that in D:L cycle, after both 4 and 8 weeks of acclimation (0.5 ± 0.1°C, P < 0.001 and 0.3 ± 0.03°C, P < 0.001; respectively). In saline-injected hamsters acclimated to L:D cycle, mean increase in T_{BAT1} (0.3 ± 0.1°C) was similar to the increase recorded in animals acclimated to D:L cycle for 4 weeks (0.2 ± 0.04°C). In both experiments, changes in
BAT1 were larger than after 8 weeks in D:L cycle (0.1 ± 0.05°C, P < 0.001).

Changes in mean ambient temperature (PTa) were also closely correlated with photoschedule and type of injection (two-way ANOVA: F(2,176) = 8.84, P < 0.0002; Fig. 3B). Changes in mean PTa after saline administration did not differ between experiments (0.2 ± 0.4°C in L:D cycle, 0.15 ± 0.2°C in D:L cycle—4 weeks, and 0.4 ± 0.3°C in D:L cycle—8 weeks, P = 0.97). In NA-injected hamsters, the largest decrease in PTa was recorded in L:D cycle (mean for all injections: −5.8 ± 0.4°C); it was larger than the mean decrease in D:L cycle, after 4 and 8 weeks of acclimation (−4.1 ± 0.4°C, P < 0.001 and −0.6 ± 0.3°C, P < 0.001; respectively). The difference between mean changes in PTa after NA injections in hamsters acclimated to D:L cycle for 4 and 8 weeks was also significant (P < 0.001).

DISCUSSION

Twelve weeks of exposure of hamsters to 12 hr of light (irrespective of photoschedule reversal) led to the gradual attenuation of regulatory NST. The mean increase in BAT temperature (TBAT1) and mean decrease in preferred ambient temperature (PTa), recorded after NA injections, were largest after 4 weeks of acclimation to L:D cycle and least after 8 weeks of acclimation to D:L cycle. One might relate this reaction to the habituation to repeated injections. However, this is not the case because there was no difference in the response to injections between hamsters used for the first time and those re-tested. Furthermore, hamsters acclimated to cold and a short photoperiod for...
6 months, and then injected with NA and saline every 4 weeks did not exhibit such a gradual decrease in BAT capacity for NST (Jefimow et al., 2004b).

The gradual decrease in BAT capacity for NST observed in the hamsters seems to be a natural consequence of the photoperiodic history of the animals. For the Siberian hamster, a 12L:12D photoperiod was regarded as short or intermediate, between long and short ones and vice versa (Hoffmann, '73). The present experiments began in March, when the natural photoperiod was about 12L:12D. Despite the additional 12 weeks of exposure to 12 hr of light, hamsters changed to their summer status characterized by low capacity for NST, grey fur and increased body mass (Hoffmann, '73; Heldmaier et al., '82). In the present experiments, the hamsters significantly increased body mass with the time of acclimation (P < 0.0001) and at the end of experiments, they were about 30% heavier than at the beginning. Concomitantly NST capacity decreased. These two facts clearly indicate that at the time of experiments hamsters were photorefractory and that they possessed a memory of the previous photoperiodic conditions. Despite prolonged exposure to 12 hr of light and photoschedule reversal, the hamsters not only continued to change to their summer status but they were also able to fully acclimate to the reversed photoschedule: daily rhythms of body temperature, preferred ambient temperature and NST capacity were entrained to the new D:L cycle. Since intermediate photoperiod (12L:12D) occurs in a temperate zone twice a year (in spring and autumn), the direction of changes in day length is very important. Lengthening or shortening photoperiod provides information (encoded in the melatonin signal) about the forthcoming time of year and allows hamsters to prepare for winter or summer conditions. However, critical day length plays a very important role only during the photosensitivity phase, while photorefractoriness is controlled endogenously and does not depend on critical photoperiod (Gorman and Zucker, '98; Goldman, 2001). Photosensitivity in autumn and photorefractoriness in spring have an adaptive value and are very important for photoperiodic mammals in their annual rhythms of reproduction and thermoregulation (Goldman et al., 2004). The Siberian hamsters breed during late spring and summer when food is abundant and ambient temperature is relatively high. On the contrary, a gradual increase in NST capacity and decrease in body mass in autumn ensure winter survival. Taken together, the gradual decrease in the capacity for NST and increase in body mass recorded during this study reflects hamsters’ entering summer status despite being housed in constant environment. Physiological, morphological and behavioural changes observed during the time of photorefractoriness cannot be prevented by continuous exposure to a short photoperiod or melatonin infusion (Heldmaier et al., '81; Lynch and Puchalski, '86; Puchalski and Lynch, '86; Schlatt et al., '95; Goldman, 2001; Goldman et al., 2004) or by photoschedule reversal studied in the present experiments.

Siberian hamsters are nocturnal (Scribner and Wynne-Edwards, '94; Ross, '98; Ruf and Heldmaier, 2000). In hamsters acclimated to L:D cycle, a clear-cut rhythm of BAT temperature (T_{BAT0}), with higher values at night, was recorded. After 4 and 8 weeks of acclimation to a reversed photoschedule, the hamsters’ daily rhythms of T_{BAT0} and preferred ambient temperature were reversed and entrained to the new conditions. BAT temperature was always lower and preferred ambient temperature was higher by day than by night. The re-entrainment of body temperature (T_b) rhythm after reversal of L:D cycle was also recorded in Wistar rats (Zerath et al., '94). Rhythmic T_b persisted for 2 days after photoperiod reversal, and was fully entrained to the new photoperiod within 7–9 days following reversal. Thus, it is not surprising that after 4 weeks under a reversed photoschedule, BAT temperature rhythm of Siberian hamsters was completely re-entrained to the new D:L cycle.

The results of the present experiments also confirmed our prediction that BAT capacity for regulatory NST depends on the actual level of T_b. In hamsters acclimated to L:D cycle, we recorded large daily variations in the response to NA. The NA-induced increase in BAT temperature (T_{BAT1}) was inversely correlated with BAT temperature before injection (T_{BAT0}); that is, when T_{BAT0} was high, NA elicited a smaller increase in T_{BAT1} (P < 0.002). The most pronounced effect of NA was recorded after injection during the day and during the second part of the night. A similar pattern was found in previous studies on Siberian hamsters acclimated to cold (T_a = 10°C) and a short photoperiod (8L:16D), when abdominal T_b or preferred ambient temperature were measured (Jefimow et al., 2000, 2003). In the present study, the increase in T_{BAT} after NA administration was also correlated with decrease in PT_a (P < 0.001). This
correlation suggests that NA-induced changes in $PT_a$ reflect the intensity of NST in BAT. When the increase in $T_{BAT_1}$ is large, animals may choose a lower $T_a$ in order to dissipate excess heat. This preference for a lower $T_a$ may prevent them from overheating, and indicates the importance of behavioural thermoregulation that supports autonomic reactions in maintenance of $T_b$ at the level determined by their set point (Gordon, '93). Selection of lower $T_a$ recorded during the $\alpha$ phase of the 24-hr cycle may prevent an excessive elevation in $T_b$ (Gordon, '90, '94).

We also predicted that daily rhythm of NST would follow daily rhythm of body temperature. However, after 4 weeks of acclimation to a reversed photoschedule, BAT capacity for NST decreased, and rhythmicity of NST capacity disappeared. Moreover, there was no correlation between $T_{BAT_0}$ and $T_{BAT_1}$ after NA injection. This implies that the NST rhythm is not a direct result of $T_b$ rhythm, although they are related.

Rhythmicity of the response to NA appeared again after 8 weeks of acclimation to D:L cycle. The maximum effects of NA were recorded after the daytime injection (CT 4 and CT 8), when $T_{BAT_0}$ was low, similar to what was observed in L:D cycle. Due to the hamsters changing to the summer status, with lowered NST capacity, rhythmicity of NST in BAT was much more difficult to detect. However, a significant inverse correlation between $T_{BAT_0}$ and $T_{BAT_1}$ after NA injection ($P<0.001$) indicates re-entrainment of the NST rhythm. We are not able to say exactly at which time this re-entrainment was complete, but it occurred between the fifth and eighth week of acclimation to the reversed photoschedule. Thus, the establishment of a new reversed rhythm of NST takes more time than the establishment of a body temperature rhythm. This rhythm seems to be more conservative than $T_b$ rhythm, and it does not seem to be responsible for generation of daily fluctuations in $T_b$.

A daily rhythm of the response to exogenous NA was found in golden and common spiny mice (Kronfeld et al., '94; Haim and Zisapel, '99). Maximum NST capacity was recorded when body temperature and oxygen consumption were at their circadian minimum. In contrast, in wood mice, the maximum capacity for NST was recorded when body temperature was high (Haim et al., '95). When NST is already activated (e.g., during the $\alpha$ phase), further stimulation with exogenous NA may elicit little effect, since endogenous NA occupies the adrenergic receptors (Kronfeld et al., '94; Jefimow et al., 2003). Daily variations in NST capacity may also result from the daily rhythms of heat production and heat loss since both of them undergo circadian variations, contributing to the generation of the $T_b$ rhythm (Aschoff, '83; Refinetti and Menaker, '92). Since heat loss is promoted during the $\alpha$ phase of every 24-hr cycle (Aschoff, '81), the increase in $T_{BAT}$ after NA injection was smaller at night or when the hamsters were active. These two rhythms, heat production and heat dissipation, as well as daily rhythm of activity might contribute to the daily variations in the response to saline that reflects stress of handling and injection. The magnitude of changes in $T_{BAT}$ after saline might also result from the fact that stress-induced hyperthermia depends on the time of day (Peloso et al., 2002).

We conclude that BAT capacity for NST has a daily rhythm in Siberian hamsters. After photoschedule reversal, the re-entrainment of body temperature ($T_b$) and preferred ambient temperature ($PT_a$) rhythms precede re-entrainment of the NST rhythm. NST rhythm seems to have lower plasticity and adjusts to the new environment slower than $T_b$ and $PT_a$. The present experiments provide evidence for the idea that BAT capacity for NST depends on $T_b$; that is, there is an inverse correlation between NST capacity and $T_b$. We suggest that, in Siberian hamsters, during the $\rho$ phase of the day, when $T_b$ is maintained at a lower level and obligatory NST is low, a high capacity for regulatory (BAT-generated) NST might ensure the possibility to rapidly elevate $T_b$.

To the best of our knowledge, this is the first report describing the hamsters’ ability to entrain their rhythms of body temperature, preferred ambient temperature and NST capacity to the reversed photoschedule during the time of photorefractoriness. The present experiments began in March, when hamsters were photorefractory. In the course of acclimation, they continued to change to their summer status and reversal of photoschedule did not prevent gradual, annual changes in body mass and NST capacity. Daily rhythms of body temperature, preferred ambient temperature and NST capacity were fully entrained to the new lighting conditions. These rhythms are determined not only by prevailing photoperiod but also by day length previously experienced. Our results also indicate that photoperiodic history of animals, prior to their use in experiments, is of great importance and must always be taken into account when
studying seasonal rhythms under constant laboratory conditions.

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LITERATURE CITED


